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PHYTOPATHOLOGY

VOLUME XIV NUMBER 6

JUNE, 1924

SPORE GERMINATION OF USTILAGO STRIAEFORMIS

WILLIAM HAROLD DAVIS[1]

WITH PLATES XIV–XVI AND ONE TEXT FIGURE

During each decade of the last half century, some scientific investigator has endeavored to germinate the smut spores of *Ustilago striaeformis* (Westd.) Niessl which parasitizes some of our most important economic grasses. Published observations of these investigators show that conditions for the successful germination of the smut spores of this fungus have not been understood and, for this reason, there has been more or less speculation as to whether the organism is an Ustilago or a Tilletia. Furthermore, in later years, mycologists and pathologists have assigned the smut organisms on our common grasses to one morphological species of Ustilago, *U. striaeformis*, regardless of the grass host parasitized. This was probably due to the fact that the symptoms of the disease and the sizes and echinulated surfaces of the spores are comparatively the same on each of the different grass hosts. Even if there is only one morphological species, the problem of physiological races and the life history of the organism cannot be solved until the conditions for the germination of the spores have been definitely determined.

This investigation was undertaken for the specific purpose of determining the natural conditions under which the spores of this smut germinate, the nature of the germination, the genus of the fungus on each host investigated.

THE DISEASE

The leaf smut of timothy appears on the leaves and stems of grasses as long, narrow, dark striations which split open and shred the leaf blades into long strips. (Plate XIV, fig. 1.) The writer has observed these striations on rhachides, rhachillae, glumes, lemmata, palea, stamens and ovaries of timothy and redtop plants. Ovaries of timothy and redtop are sometimes filled with spore masses similar to smutted ovaries in the closed smuts of cereals.

[1] The writer wishes to express sincere appreciation to Professor L. R. Jones for his inspiration and assistance during this investigation and in the preparation of the manuscript; to Dr. J. J. Davis and Dr. J. G. Dickson for suggestions and criticisms.

(Plate XIV, figs. 2, 3, 4 A–B.) Also, there is a noticeable dwarfing of the diseased culms which average about six tenths of the height of healthy ones. Diseased culms seldom bear seeds but when they do, the yield is about one fourth the number commonly observed on healthy plants.

The striations and smutted ovaries are filled with spores,[2] the surfaces of which are covered with a thin, hyaline wrinkled membranous tissue appearing microscopically as a fine network. In this network are spines or aciculae less than one micron in length and from thirty to seventy on one facies of a spore. Underneath the network is a rather thick colored layer, the exosporium, which covers a thicker, hyaline layer, the endosporium. No germspores were observed in the spore walls. (Plate XIV, fig. 4, F–G.)

The measurements of 50 fresh smut spores removed from dried leaves of different hosts and mounted in water follow:

Timothy	8.4×11	microns
Redtop	9.3×11.1	"
June grass	8.6×10.2	"
Orchard grass	10.7×11.5	"

Limits for all four hosts, $8–11 \times 10–12$ microns.

The writer has collected *U. striaeformis* on the following hosts: redtop, *Agrostis palustris* Huds.; timothy, *Phleum pratense* L.; June grass, *Poa pratensis* L.; orchard grass, *Dactylis glomerata* L. Although this smut has been reported as a parasite on about 40 different species of the Gramineae, this investigation deals with the smut found on timothy, redtop, June and orchard grasses only. An examination of reports from various countries and states would lead one to believe that the disease is principally confined to the humid central portion of the north temperate zone between the north parallels of 30 and 50 degrees. Different reports of losses within this area vary. Clinton (7) cited an instance where the seed production was reduced from 30 cwt. to 70 cwt. In 1905, he reported the parasite especially harmful to lawn grass which observations confirmed Pammel's of 1893–1901. Osner (19) found the loss as high as 30 per cent, while Johnson and Haskell (16) reported a slight loss in Minnesota, Wisconsin, Idaho, and a trace in Iowa. The writer's observations verify the above reports. The most critical period for infected plants occurred during periods of drought when over 90 per cent of the infected plants succumbed. Thus most of the losses may be placed in four classes: (1) reduction in yield of hay as indicated by shredding of diseased leaves; (2) reduction in amount and quality of seed; (3) lowering the carrying capacity of pastures; (4) injury to lawns.

[2] In conformity with common usage, the term smut "spore" is here used synonymously for chlamydospore.

METHODS

Spore germination tests were made of smutted materials from timothy, redtop, June and orchard grass and then portions of each of these materials were placed in wire packets stored outdoors. The remaining portions were placed in corked vials lined with wet filter paper and stored in the laboratory. In addition, some infected plants were marked in the field; others removed from the field, transplanted into pots and grown in the greenhouse.

The mycelium in all host tissues was well differentiated by staining with Flemming's safranin-gentian-violet-orange G., employing a 120:2:1 minute staining schedule. The germinated spores were stained according to a method described by the author (11).

The agars, solutions and decoctions were prepared according to formulae described by Duggar, Jensen (15), Guillermond and Tanner (13).

The percentage of spore germination was obtained by counting 100 spores in an average field under the 8 mm. objective and, at the same time, recording the number germinated. The average germination obtained from a number of such counts was recorded as the percentage of germination for the sample.

GERMINATION OF FRESH SPORES

Brefeld (3, 5) showed that some smut spores which germinate sparsely or not at all in water, germinate and sporulate satisfactorily when incubated in nutrient solution. In an endeavor to find a medium suitable for germination, smut spores from timothy, redtop, June and orchard grass were incubated in various solutions and on different substrata under different conditions of air, heat, light and moisture. The spores were removed from infected green leaves, from infected leaves in the bud not yet unrolled and from old dried leaves growing in the field and in the greenhouse.

A list of materials employed follows: sterilized, unsterilized and filtered water from melted snow, from a tap and from a cistern; juices of potato tuber; rhubarb petioles, cherry, apple, grape, pear, peach, lemon, orange, tomato, plum and prune; decoctions prepared from different parts of the grass hosts (roots, culm, leaf, blossom and seedling), prunes, raisins and manures; water solutions of nectar from the Easter lily, aphid "honey dew," honey, molasses, renin, pepsin, trypsin, saliva, vitamine B, ammonia and wine; nutrient solutions such as Cohn's, Pasteur's, Laurent's, beerwort, beef broth; agars as prune, potato dextrose, potato, corn meal, oat, water and beerwort; substrata composed of plant parts as roots, stems, leaves, flowers, seeds, seedlings and decayed parts of the hosts; also on damp filter paper and pith laid on acid, alkaline and neutral soils and submerged in the soil water; solutions of acids such as oxalic, malic, citric, tartaric, sulphuric, nitric and hydrochloric.

In fifty spore germination tests with water from different sources, less than ten germinated spores from timothy were observed and those in two cultures. Four repeated trials with the solutions apparently favorable in the first trial, showed the smut spores from June grass germinated less than 0.5 per cent in the decoctions but germinated spores were also found in the distilled water checks. Smut spores from June grass also germinated in a 0.2 per cent citric and malic acid solutions adjusted to + 15 and + 20 Fuller's scale. However, a repetition of this experiment employing two-months'-old spores from timothy gave good spore germination from + 5 to + 16. This test together with others showed that the age of the spore influenced the results and was a greater factor in germination than the acidity of the solution employed.

Thus it would seem that favorable germination of fresh smut spores from timothy, redtop, June and orchard grasses is not entirely dependent on any one of the following factors: (1) age of the infected *green* leaf from which the spores may be removed; (2) the kind of solution, decoction, plant part or other substrate on which the spores are placed; (3) intermittent freezing and thawing; a certain temperature or varying amounts of air and moisture.

SPORE DISINFECTION

The surfaces of after-ripened spores from timothy and orchard grass were sterilized in a four per cent and a one per cent copper sulfate solution, a 1 : 1000 mercuric bichloride solution and sterile distilled water acting for intervals varying from one to fifteen minutes. The spores were separated from the liquids by methods commonly used for such work employing a centrifuge and the necessary aseptic precautions. After sterilizing and centrifuging, the spore samples were transferred to Cohn's, Laurent's and Pasteur's solutions with and without a five per cent molasses solution; beer-wort, manure extract, a 10 per cent strained honey solution, sterile water; to agars as potato, nutrient, onion, beerwort, oat and Czapek's. The H-ion concentration of Czapek's agar was varied as follows: pH 4.2, 4.8, 5, 6, 6.2, 7.1, 8.4.

The results showed that either a four per cent or a one per cent copper sulfate solution acting on the after-ripened spores from timothy and orchard grass for five minutes prevented germination. A few spores germinated after being treated for three minutes in the copper sulfate solutions but the spore surfaces were not always sterilized. In no case did the treated spores germinate over five per cent, while the checks germinated 50 to 90 per cent.

Spores treated two minutes in the mercuric bichloride solution failed to germinate while the untreated checks germinated 30 to 90 per cent. This

solution like the copper sulfate solution proved unsatisfactory because a sufficient number of the spores did not remain viable after the briefest period for which the spores could be conveniently treated.

Cohn's, Pasteur's and Laurent's nutrient solutions, manure decoctions and beerwort did not cause abundant formation of typical sporidia. The promycelium grew and branched in the honey solution but typical sporidia failed to develop.

On agars, a few promycelia grew in length about twice the spore diameter, after which growth ceased. Several promycelia branched but failed to form actively growing saprophytic mycelium during the 60 days they were under observation. Oat agar gave the best results as the promycelia were slightly longer. Varying the pH value of Czapek's agar seemed to have no beneficial effects on the growth in length of the promycelium and formation of typical sporidia.

AFTER-RIPENING AND GERMINATION

Brefeld (4), Paravicini (27), Kniep (17), Anderson (1) and Bauch (2) recognized the importance of an after-ripening or a rest period for smut spores previous to their spore germination and infection experiments. Fischer von Waldheim, Pammel, Clinton, Osner and others failed to successfully germinate the spores of *U. striaeformis* even when collected from materials of different ages. Osner (19) endeavored to find an after-ripening period for these smut spores by placing smutted portions of timothy between wire screens which were then stored outdoors. However, he was unsuccessful in germinating spores from his stored materials. Thus it can be stated that no after-ripening period for the spores of *U. striaeformis* has been observed and described.

For the purpose of finding whether the spores of *U. striaeformis* have an after-ripening period which might have been overlooked by the previous investigators, highly infected green leaves were collected from timothy, redtop, June and orchard grass growing in the field. Samples of each of these collections were stored in the laboratory and outdoors as previously described. Weekly spore germination tests were made from each sample of stored materials which represented collections made during different months of the year as those from timothy were collected and stored during the months of February, April, June, July, August and October. All data for each stored spore sample was tabulated but lack of space prohibits the presentation of this tabulation showing the name of the host, where the materials were stored, date when the spores were placed to incubate, date and per cent of maximum germination, after-ripening period in days, date for the cessation of germination and period of spore viability. However, a summary of these observations is to be found in table 1.

TABLE 1—*A summary of spore germination tests showing the periods of after-ripening,*[1] *viability,*[2] *and germinability*[3] *for the smut spores of U. striaeformis*

Grass hosts	After-ripening conditions	Average after-ripening periods Days	Average periods of		No. of samples
			viability Days	germinability Days	
Timothy	Laboratory	206	287	70	5
	Outdoor	302	341	72	5
Redtop	Laboratory	302	347	71	6
	Outdoor	250	303	84	2
June	Laboratory	197	341	120	3
	Outdoor	250	325	117	3
Orchard	Laboratory	251	306	70	3
	Outdoor	254	319	64	3

[1] *The after-ripening period* is used synonymously with rest period, and dormant period. This period has usually been considered as the interval elapsing between spore formation and spore germination, but as the time of spore formation could not be accurately determined, the after-ripening period has been considered here as the interval which elapsed between the death of the green host tissues in which the spores were formed and the date of a test showing the maximum percentage of germination. The host tissues were considered dead on the same day as removed from the plant and stored.

[2] *Period of viability:* The period of after-ripening and the period of germinability of the smut spores—longevity of the spores.

[3] *Period of germinability:* Elapse of time from the after-ripening period to the natural death of ungerminated smut spores.

From table 1 it is to be noted that the spores of *U. striaeformis* from timothy, redtop, June and orchard grass germinated satisfactorily after passing through an after-ripening period of approximately 250 days. Experiments showed that the after-ripening period for the spores in green materials stored in the spring was longer than for similar materials stored in the fall. Also, the after-ripening period was shorter for spores in dead materials than for those in green materials stored at the same time and under similar conditions. Thus after-ripening begins with the spores in the host. One lot of spores collected on July 3, from timothy and redtop, germinated 30 per cent but after storing for 28 days, 90 per cent. So by chance, during the summer, one might find in dead, overwintered leaves spores which are after-ripened and germinable.

Five different samples of smutted materials from each of three hosts were subjected to the fumes of chloroform for one minute then submerged in a 10 per cent citric acid solution for five minutes, washed and set to after-ripen. This treatment decreased the after-ripening period of spores from timothy about thirty days; from redtop, 18 days, and from June grass, 20 days. However, the period of after-ripening following this treat-

ment depended somewhat on the temperature at which the spores were stored, about 20° C. being the most favorable. One lot of treated spores stored at 8.5° C. after-ripened after 332 days; a second lot at 16° C. after 200 days and a third lot at 19.2° C. after 186 days, while other lots set at 5° and 30° C. failed to show after-ripening. Spores in the untreated checks also failed to after-ripen at 5° and 30° C.

The average period of viability for the smut spores from all four hosts was 320 days. However, one lot of spores in green leaves of redtop remained viable in storage for 460 days, the maximum period observed. The minimum period of viability was 124 days and this was for spores in a sample of old smutted timothy leaves collected in March and stored in the laboratory. These spores had been formed no later than during November of the previous year or 150 days previous to storage thus giving a viability of about 300 days or nearer the average, 320 days.

When the smut spores were once after-ripened and stored in a humid atmosphere at 20° C. they retained their germinability for approximately 75 days except the spores from June grass which retained germinability for 120 days. This period of germinability was difficult to ascertain with absolute certainty as each test was made with a separate lot of spores and various lots were under different conditions even in the same leaf. Observations showed that the period of germinability for smut spores formed in the fall was the same as for those formed in the spring. However, Bauch (2, p. 245) observed that the spores of *U. longissima* retained their germinability about 120 days but spores formed in the fall remained viable longer than those formed in the spring.

Thus the period of germinability for the spores from timothy, redtop and orchard grass was approximately 75 days and for smut spores from June grass, approximately 120 days.

Experiments were conducted to determine the effects of air-drying on the periods of viability and germinability. Smutted timothy plants in herbarium materials which had been stored for 1 to 20 years were stored for the spores to after-ripen. The results showed that materials thus dried in herbarium packets for one year or more were non-viable. One lot of smut spores from timothy remained viable under dry-air conditions for 140 days and a lot from redtop, 67 days. These were the maximum periods observed for spores from these hosts. If after-ripened spores were air-dried, they ceased germination, and thus their period of germinability was shortened. While, after-ripening, the spores from all four hosts seemed to withstand arid outdoor conditions fairly well but when after-ripened they quickly succumbed to similar arid conditions.

Observations of conditions for germinating spores of *U. striaeformis* removed from all four hosts seemed to warrant the following statements:

1. Spores from different pustules within a leaf vary in the time of germination and length of viability.

2. If old diseased leaves and green diseased leaves are placed under proper conditions for spore after-ripening the spores in the old leaves after-ripen and germinate first.

3. Smut spores in leaves overwintered at the base of a culm after-ripen sooner than spores removed from green leaves on the same plant collected in the following spring or summer.

4. Spores from the base of a leaf sometimes germinate before those from the tip.

5. Storage in a moist atmosphere favors after-ripening.

6. Alternate drying and moistening the materials in the early periods of after-ripening seems favorable to after-ripening.

7. Drying after-ripened spores for a period longer than seven days greatly decreased the viability, while drying them one to two days stimulated germination in three cases.

8. Smut spores from infected timothy stored in herbarium packets for 140 days and from redtop 67 days, then set to after-ripen, germinated 40 per cent showing that drying spores soon after spore formation does not impair or diminsh the viability as much as drying them during the after-ripening period.

9. Low temperatures delay after-ripening; 20° C. being the most favorable.

10. Contaminations in spore germination cultures are generally unfavorable to germination. After-ripened, viable spores germinate well when on the surface and when submerged in water; also, when within decayed host tissues and "spore balls."

11. Fresh smut spores treated with chloroform and citric acid after-ripen and germinate three to four weeks earlier than untreated checks.

12. The approximate periods associated with the germination of the smut spores from timothy, redtop, June and orchard grass were observed as follows: after-ripening, 250 days; viability, 325 days; germinability, 75 days, except June grass with a germinability of 120 days.

MORPHOLOGICAL FEATURES OF GERMINATING SPORES FROM TIMOTHY, REDTOP, JUNE AND ORCHARD GRASSES

Pammel (26) stated that the spores of *Tilletia striaeformis* (Westd.) Magnus (*U. striaeformis*) germinated readily and in the same way as those of the stinking smut of wheat, *Tilletia foetens* (B. and C.) Trel., but Clinton found the spores germinated with difficulty and the promycelia of germinated spores from redtop bore cross-walls as those in the genus Ustilago.

Osner (19) reported cross-walls and "well developed clamp connections" in the promycelia on two slides where a few spores from redtop germinated. A review of the publications by previous investigators shows there remains some doubt regarding the type of germination of the spores of *U. striaeformis* on timothy, redtop, June and orchard grasses.

Germinating spores from timothy (Plate XV).—After properly incubating after-ripened smut spores from timothy for 12 hours, rifts appear in the exosporium (Plate XIV, Fig. 4, F) and later a hyaline germtube, the promycelium, emerges. Promycelia were two to six microns in diameter, of varying lengths from 30 microns to one millimeter when submerged in liquids and filled with granular, vacuolate, deeply staining protoplasm. The wall of the promycelium is hyaline and continuous with the spore membrane.

After 24 to 48 hours incubation, the granular protoplasm advances and assembles in the tip of the promycelium leaving a hyaline portion between it and the spore. Slides bearing stained spores which had been incubated 48 to 72 hours showed the promycelium bears cross-walls and one to five branches or lateral sporidia which generally appear near the cross-walls on the side towards the spore. The cross-walls, number and length of lateral sporidia were aided by the following factors: (1) spores germinated at the termination of their after-ripening period; (2) germination at low temperatures, 12° C.; (3) acid solutions; (4) nutrient solutions such as apple juice, honey, Cohn's and Laurent's. In some old cultures, lateral sporidia developed in these nutrient solutions branched, bore cross-walls and duplicated the original promycelium and its parts. This was especially noticeable in honey solutions.

So many unsuccessful attempts were made to discover conditions under which promycelia would form typical sporidia that the author believes typical sporidia seldom, if ever, form on the promycelia of germinating smut spores from timothy. The types of germination for these spores seemed to fall in the same category as those of *U. tritici, U. dura* and *U. nuda*, cited by Paravicini (27) as "the mycelium is the whole state of the matter." For this reason, Herzberg (14) assigned organisms with spores germinating in this manner to the genus Ustilagidium.

Brefeld (3) and Bauch (2) showed that germinating spores of *U. longissima* formed primary sporidia which under favorable conditions, either fused with other sporidia, budded or developed into mycelial threads. Paravicini (27) also showed that species of Ustilago form these buds or secondary sporidia which may fuse forming a conidium and this in turn develop a mycelial thread.

Primary sporidia were sometimes cut off the tips of a short promycelium-like growth protruding from the spore or remained within the spore walls.

These were observed especially among spores from timothy after-ripened during an unusually short period, treated with organic acids previous to germination, germinated in a weak solution of organic acid at low temperatures, as 15° C., or at high temperatures of 29° to 30° C. (Plate XV, Fig. 8–16). A unicellular primary sporidium sometimes developed, dividing "in situ" to form a necklace of cells which gave the appearance of a promycelium composed of several short uninucleated cells (Plate XV, Fig. 16). In weak organic acids and nutrient solutions, some primary sporidia formed buds which appeared uninucleated in stained slides. In three cases primary sporidia fused, the contents of one passed into the other and formed a conidium which developed a mycelial thread. Primary sporidia were observed emerging from the smut spore and budding.

Primary sporidia varied from 3 to 16 microns in length and from 1.5 to 6 microns in width. Most of those observed in stained slides contained two nuclei and granular protoplasm with vacuoles. On stained slides, it was difficult to distinguish primary sporidia from promycelia as the final outcome of germination could not be further observed without developing special technique. (Plate XV, Fig. 8–10.) Some of the apparently shorter primary sporidia were uninucleate. The above observations of germinating smut spores from timothy may be summarized.

1. Typical germination in water produces a long promycelium bearing one to five lateral sporidia, branched or unbranched, with the protoplasm assembled mostly in the tip or tips.

2. The promycelia and lateral sporidia sometimes possess cross-walls, branches or lateral sporidia.

3. Primary sporidia are formed directly from the spore or within spores similar to those formed in *U. longissima*.

4. Typical sporidia are seldom if ever formed.

5. Sporidia sometimes fuse forming conidia which develop mycelia.

6. Buds are formed on primary sporidia but sometimes emerge directly from the germinating smut spore.

7. The typical germination of these smut spores is similar to the germination of the spores of *U. tritici*, *U. nuda* and *U. dura*.

Germination of spores from redtop (Plate XVI, Figs. 1, 2, 4).—Smut spores from redtop were after-ripened, incubated, germinated and stained by methods previously described for the smut spores from timothy.

The granular protoplasm sometimes advanced to the tip of the promycelium; other times, the content was granular and bore one to five branches or lateral sporidia. The promycelia and sporidia bore cross-walls. Primary sporidia sometimes protruded from the spore but at other times remained within it. Uninucleated buds were formed from spores, primary sporidia and other buds.

Thus the germination of the spores from redtop was of the same type and agreed favorably with the partial description by Clinton and Osner. However, no clamp connections between promycelial cells were observed in the numerous spore germination cultures. As far as could be determined, the germination of the spores from redtop was identical with that previously described for spores from timothy.

Germinated spores from June grass (Plate XVI, Figs. 3, 5–7).—Observations of numerous spore germination cultures showed that the typical germination for most of the smut spores from June grass was similar to the germination already described for the smut spores from timothy and redtop. However, in two lots of germinating spores, the promycelium together with its lateral sporidia separated from the spore and a disturbance of the liquid caused some of the sporidia to separate from the promycelium. In different cultures, two of these free sporidia came in contact, fused either laterally or apically and formed a conidium which developed a mycelial thread or buds.

When after-ripened smut spores were treated with acids and incubated in weak solutions of organic acids or in water, primary sporidia sometimes developed acropically and apparently divided in place giving the appearance of a promycelium composed of two to five short cells. These primary sporidia sometimes bore buds laterally and apically. (Plate XVI, Fig. 3.) As far as determined, most of these buds were uninucleate when formed.

Thus the common type of germination for the smut spores from timothy, redtop and June grass was similar with the additional fusion of lateral sporidia observed in the germinating smut spores from redtop.

Germinating smut spores from orchard grass (Plate XVI, Figs. 8–10).—While fewer cultures of germinating smut spores from orchard grass than from timothy were under observation, yet there were sufficiently large numbers to show no decided, external differences in the type of germination However, some lateral sporidia formed exceptionally long, septate hyphae. Otherwise, the common type of germination for the spores from timothy, redtop, June and orchard grass was the same.

RELATION OF TEMPERATURE TO GERMINATION

The influence of temperature on the germination of these smut spores was determined by incubating the spore samples in an Altmann incubator where the temperatures of the compartments ranged from 6° to 20° C., at approximately 2° intervals. For temperatures ranging from 20° to 38° C. incubators and electric ovens were employed. The temperatures were read three times daily and only averages were reported in the results. Where neither the liquid nor definite temperatures are stated for the ger-

mination tests, a room temperature of about 20° C. and distilled tap water were employed.

Fresh smut spores from all four hosts were set to germinate at temperatures ranging from 0° to 38° C. and the samples interchanged from low to high temperatures. Spores that had remained outdoors during zero weather were tested for germination at the various temperatures. All results were negative and showed temperature is not the important factor for inducing the germination of the fresh smut spores.

As there was no experimental evidence on the relation of temperature to the germination of spores of *Ustilago striaeformis*, after-ripened spores were incubated at the different temperatures stated above to determine the minimum, optimum and maximum temperatures. Data were averaged from three trials and the results are shown in figure 1.

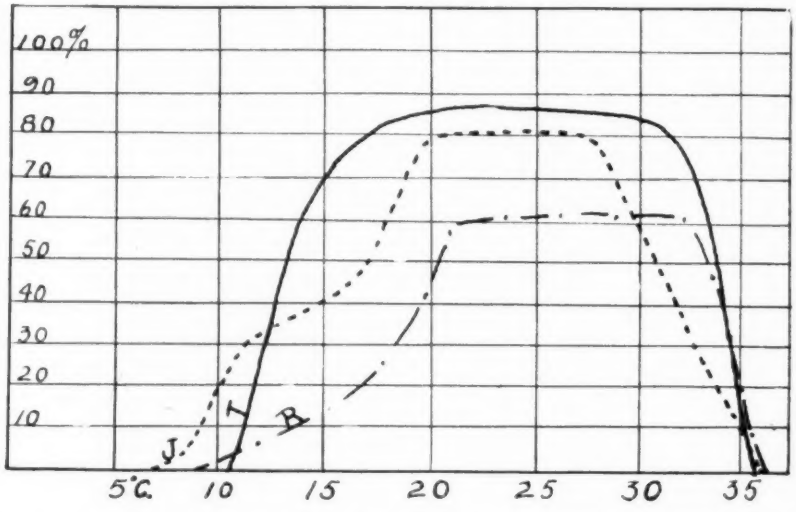

Fig. 1. Curves showing the effect of different temperatures on the percentage of germination of after-ripened smut spores from three different hosts; timothy (T), redtop, (R), and June grass (J).

The following conclusions were drawn from these germination tests:

1. The after-ripened smut spores from timothy show a minimum germination at 12° C.; optimum, at 25° C., with a range from 20° to 30° C., the maximum being 37° C.

2. In general, the cardinal temperatures for the germination of the smut spores from timothy, redtop, and June grass may be stated: minimum, 7° C., optimum, 22° C., maximum, 35° C.

3. After-ripened smut spores from orchard grass were set to germinate at 8°, 22°, and 38° C. The results showed that the cardinal temperatures

for the germination of the smut spores from orchard grass are about the same as those stated above for timothy, redtop, and June grass.

TAXONOMY

Only a brief history of the taxonomy of this pathogen need be given here as it has been reviewed by Osner (19) and others. The following is a list of the more important synonyms for the fungus as it has been described on various hosts:

Ustilago striaeformis (Westd.) Niessl

1. *Uredo longissima* var. *holci* Cesati. Klotz. Raben. Herb. viv. Mycol. (No. 1498). 1850.
2. *Uredo striaeformis* Westd. Acad. Roy. Belg. Bul. **18**: Ser. 2, 406. 1851.
3. *Tilletia de Baryana* Fischer von Weldheim. Raben. Fungi eur. (No. 1097). 1866.
4. *Ustilago striaeformis* (Westd.). Niessl in Hedwigia **15**: 1. 1867.
5. *Tilletia striaeformis* Oud. Oudemans in Bot. Ztg. **36**: 440. 1876.
6. *Tilletia striaeformis* (Westd.) Magnus. DeToni in Sacc. Syll. fung. **7**: No. 2, 484. 1888.
7. *Ustilago striaeformis* (Westd.) Niessl. Clinton in Ill. Agr. Exp. Sta. Bul. 57: 348–349. 1900.

From the above list of synonyms and the historical sketch of the spore germination studies as previously described, it is to be noted that definite information regarding the germination of the smut spores from timothy, redtop, June and orchard grasses had been wanting and until this information could be supplied, the genus of the organism on all four of these hosts still remained in doubt.

A brief review of the data obtained from this detailed study of germinating smut spores from timothy, redtop, June and orchard grasses demonstrates that the fungus on these four hosts is an Ustilago:

1. Sporidia form on the sides of the septate promycelia.
2. Sporidia fuse and form conidia which develop mycelial threads.
3. Primary sporidia develop directly from the spore.
4. Yeast-like buds ("Hafenpilze") may form directly from the spores, primary sporidia and from other buds.
5. The above characters are similar to those assigned the genus Ustilago by Brefeld, Rawitscher (28), Paravicini, Bauch and other investigators.
6. Similar types of germination have been reported for *U. nuda*, *U. tritici*, *U. longissima*, *U. dura* and *U. violacea*.

Thus this smut parasitizing timothy, redtop, June and orchard grass should be known as *Ustilago striaeformis* (Westd.) Niessl unless inoculations show there are different species or physiological races on the different grass hosts.

SUMMARY

Results of these spore germination studies may be summarized as follows:

1. The smut spores of *U. striaeformis* are resting spores and, under natural conditions, must pass through a period of after-ripening before germinating. The spores from all four hosts after-ripened best when stored in a damp atmosphere at about 20° C. After-ripening under laboratory conditions required an average period of 240 days; under field conditions about 265 days.

2. Alternate freezing and thawing were not necessary for germination. Smut spores which formed on all four hosts grown in the greenhouse and after-ripened at 20° C. germinated over 80 per cent.

3. After-ripening was hastened three to four weeks by exposing fresh smut spores to the fumes of chloroform for one minute, then submerging them in a 10 per cent citric acid solution for five minutes, washing and storing in a damp atmosphere at 20° C.

4. A sparse germination of spores removed from green tissues of three hosts was obtained by incubating the spores in 0.02 per cent solutions of malic and citric acids. The chemical treatment generally hastened the after-ripening process and, in some instances, seemed to stimulate immediate germination.

5. Good germination of after-ripened smut spores from all four hosts was obtained consistently by incubating the spores in water at 20° C. The after-ripened spores did not germinate on most filter paper, soil and other surfaces unless sufficient moisture was present to float the spores or cover them with a film of solution.

6. The cardinal temperatures for the germination of the smut spores from all four hosts follows: minimum, 7° C.; optimum, 22° C., and a maximum, 35° C. The minimum and maximum temperatures varied several degrees for the spores from different hosts; notably those from timothy where the minimum was at 12° C. and the maximum at 37° C.

7. The type of spore germination for all four hosts was the same. The promycelia were at first unicellular and multinucleate but under certain conditions became multicellular with four to five lateral sporidia; however, occasionally only one lateral sporidium formed on the sides. The granular protoplasm assembled in the tips of unicellular promycelia which sometimes formed lateral sporidia. Secondary spores or buds were usually formed

from conidia, primary sporidia, tips of the promycelia and other buds. Under certain conditions primary sporidia were formed directly from the spore. Lateral sporidia sometimes fused and formed conidia which developed buds and mycelial threads.

8. The germinated spores failed to form saprophytic mycelium and typical sporidia on decoctions, agars and other substrata.

9. The germination of these spores as well as the structure of the germinated spores was similar to those described for other smuts in the genus Ustilago; notably, *U. tritici* (Bjerkander) Winter; *U. nuda* (Jensen) Kellerman et Swingle; *U. hordei* (Persoon) Kellerman et Swingle; *U. avenae* (Persoon) Jensen; *U. violaceae* (Persoon) Fuckel; *U. longissima* (Sowerby) Tulasne and others.

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DESCRIPTION OF PLATES

PLATE XIV

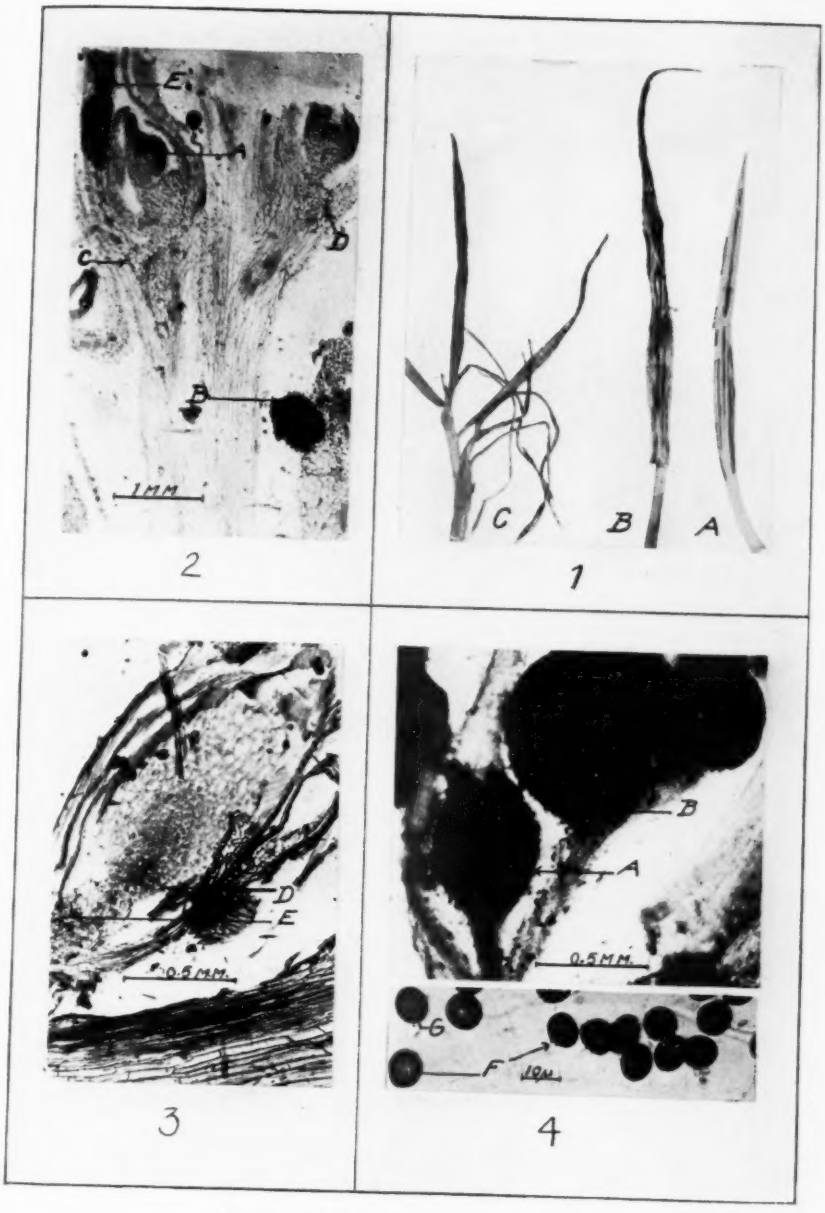
Figures 2, 3, 4, photomierographs of sections stained with safranin-gentian-violet-orange G.

Fig. 1. A. Young infected timothy leaf. B. An old infected timothy leaf showing rifts starting along the striae. C. An infected timothy plant; dwarfed and leaves shredded.

Fig. 2. Section of a redtop panicle infected with *U. striaeformis.* A. Mycelium in the ovary. B, E. Pustules of spores in glume and lemma. C, D. Mycelium in rhachis and rhachilla.

Fig. 3. Section of a timothy spikelet showing an ovary with mycelium. D. A portion of which has advanced through culm rhachis and rhachilla E, to the ovary D.

Fig. 4. A–B. Ovaries of redtop filled with spore masses. G, F. Germinated spores from timothy. F. Rifts in the spore through which the promycelium emerged. G. Spore with ecinulations.



AETIOLOGY OF THE LEAF SMUT OF TIMOTHY

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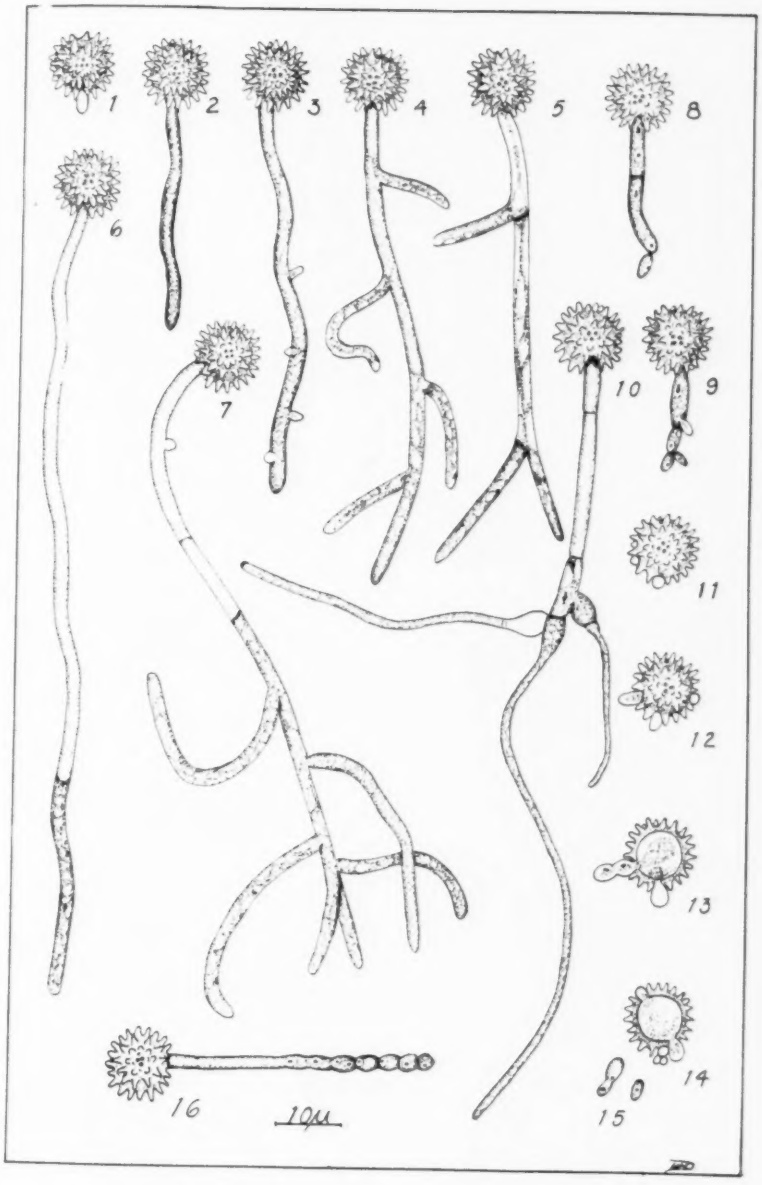

GERMINATING AFTER-RIPENED SMUT SPORES FROM TIMOTHY

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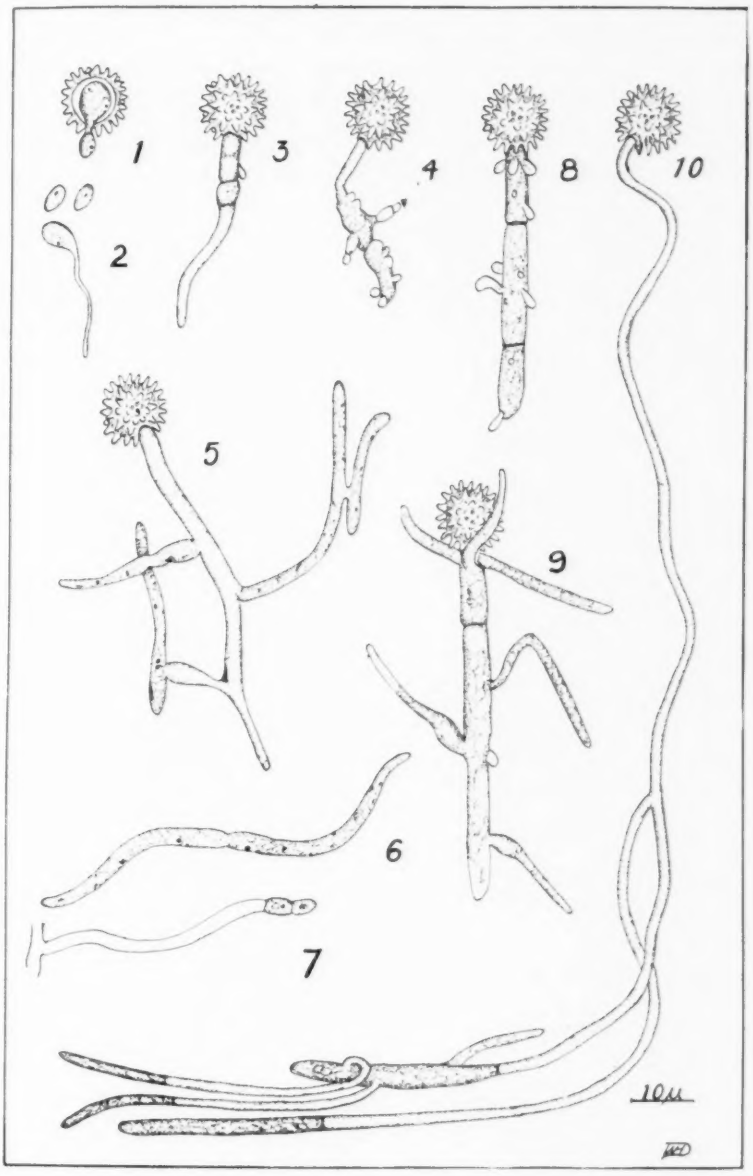

GERMINATION OF SMUT SPORES FROM REDTOP JUNE GRASS AND ORCHARD GRASS

UNIV. OF MICH.

PLATE XV

Spores incubated in distilled water at 20° C. unless otherwise stated; from camera lucida drawings.

Fig. 1. Promycelium after 12 hours incubation at 25° C. Fig. 2. Granular protoplasm filling the promycelium; 18hours incubation. Fig. 3. Lateral sporidia forming on the sides of the promycelium; 48 hours incubation. Fig. 4. Lateral sporidia after 72 hours incubation. Fig. 5. Cross-wall in the promycelium and lateral sporidium; incubated 72 hours and stained. Fig. 6. Granular protoplasm assembled in the tip of the promycelium leaving the remainder of the promycelium hyaline; 24 hours incubation. Fig. 7. Five sporidia from a promycelium previously in the condition described in fig. 6. Incubated in honey for 48 hours. Fig. 8. Primary sporidia and bud. Fig. 9. Primary sporidia and buds incubated in apple juice for 20 hours. Fig. 11–14. Buds emerging directly from an after-ripened spore incubated at 29° . Drawings show the changes at about fifteen minute intervalts. Fig. 15. Buds from stained germinated spores. Fig. 16. A row of buds on a primary sporidium. Spores incubated in a 0.05 per cent malic acid solution and stained.

PLATE XVI

Stages of after-ripened smut spores from redtop (Fig. 1, 2, 4), June grass (Fig. 3, 5–7) and orchard grass (Fig. 8–10). The general type of germination for each of these three hosts is omitted but is similar to that figured for germinated spores from timothy in plate II. Spores germinated in distilled water at 20° C. unless otherwise stated; from camera lucida drawings. Fig. 1. A bud (sporidium) emerging from the spore. From a stained specimen. Fig. 2. Buds; one with a hypha. Fig. 3. Primary sporidia; one forming a hypha, another buds. Fig. 4. Primary sporidia bearing buds. Fig. 5–6. Fusion of lateral sporidia. After-ripened spores incubated for 4 days. Fig. 7. Buds on the end of one of two fused lateral sporidia. Fig. 8. Primary sporidia bearing buds; incubated 48 hours in equal parts of Laurent's solution, molasses and water. Fig. 9. Hyphae developed from buds on primary sporidia. Incubated as described in fig. 8. Fig. 10. Exceptionally long promycelium and germinating lateral sporidia with granular protoplasm in the tips. Spores after-ripened 237 days and incubated 5 days.

REACTIONS OF SELFED LINES OF MAIZE TO USTILAGO ZEAE

H. K. Hayes, E. C. Stakman, Fred Griffee
and J. J. Christensen[1]

INTRODUCTION

Corn smut, caused by *Ustilago zeae*, cannot be prevented by ordinary control measures. The pathogene produces enormous numbers of chlamydospores; they are widely disseminated by the wind, and when they germinate in suitable nutrient solutions, such as manure decoction and soil infusions, countless numbers of sporidia are produced. The sporidia bud in a yeast-like manner, and in turn produce countless numbers of other sporidia. Thus the pathogene can exist as a saprophyte and propagate itself almost indefinitely in manure piles, compost heaps, and possibly in the soil. Both the chlamydospores and the sporidia are resistant to unfavorable conditions. The former retain their viability for as long as eight years, while the sporidia can withstand desiccation and other unfavorable conditions for several months (Piemeisel, 1917). Consequently there almost always is an abundant supply of inoculum in corn-growing regions. For this reason, crop rotation and cultural practices are ineffective, and seed treatment is valueless, because young growing tissues of corn plants of any age may be infected. Immunization therefore is the most promising method of control.

That corn smut sometimes does considerable damage is well known. But it probably is not generally realized that, even under favorable growing conditions, a fairly large percentage of ears may be completely destroyed. This was true in 1921 in a field of first generation corn crosses and their parents at University Farm, St. Paul. In this year a considerable number of ears were discarded because they were so badly infected with smut. The field on which this test was made is in a two-year rotation of corn and small grain. The percentage of ears which were discarded because of injury by smut ranged from 1.4 per cent to 8.8 per cent in six first generation corn crosses and averaged 1.9 per cent in two dent varieties, while the injury to the ears of three flint varieties ranged from 3.9 per cent to 13.4 per cent.

Jones (1918) described differences in susceptibility of strains of maize to smut attacks. The differences were hereditary and the F_1 generation of a cross between a susceptible and resistant strain was resistant while segregation occurred in the F_2 generation. Probably all plant breeders who have grown self-fertilized strains of maize have observed the fact that some strains are more susceptible than others to attacks of corn smut. Disease

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resistance in maize is one of the characters which can be best studied by the use of self-fertilized lines. Accordingly, in 1918, it was decided to determine the possibility of developing varieties of corn resistant to smut. The method of attack was selection in self-fertilized lines[2] under infection conditions.

EXPERIMENTAL METHODS AND MATERIAL

The Selfed Strains.—Although selfed strains were available, it was decided to start the experiment with commercial varieties and build up self-fertilized strains under infection conditions. Accordingly the following seven varieties of corn were selected: Rustler, Minnesota No. 13, Northwestern, Minnesota No. 23, King Phillip, Longfellow, and Squaw. With the exception of Squaw and Minnesota No. 23, which are early maturing, the varieties were adapted for the conditions in Central Minnesota. The first year each variety was grown in a row consisting of 82 plants, the plants being spaced a distance of one foot apart in the row. Two seeds were planted in each hill and the hills were thinned to a single plant basis. Vigorous plants were chosen for selfing and both infected and non-infected plants were selfed in order to determine the ease of isolating resistant and susceptible strains.

In addition to the seven varieties a few selfed strains were included. Two strains were obtained from D. F. Jones, of the Connecticut station, under the numbers 1–6–1–3–4–4–4–2–5–3–1–5 and 1–9–1–2–4–6–7–5–6–2–4 and a self-fertilized flint strain was also grown. Each of these three strains had been selfed approximately 10 generations. The two strains from Connecticut were two of those which appeared highly resistant to smut as reported by Jones.

In 1920 each selfed strain was grown in three short rows which were systematically distributed over the experimental area, and in 1921 to 1923 inclusive each strain was grown in two systematically distributed rows. From 50 to 75 plants of each strain were grown each year. At maturity the ears from 20 plants, excluding those which had been self-fertilized, were harvested. After the ears were thoroughly cured, data were taken on number of good ears, number of nubbin ears, average length of ear, average size of endosperm of seed, etc. The yield data were taken in order that a study might be made of the relation of smut infection and yielding ability of selfed strains.

Producing the Epidemic.—A considerable amount of smut was collected and stored over winter for the purpose of inducing the epidemic. The epidemic was induced by two methods. Smut spores were mixed with manure

[2] For a discussion of selection in self-fertilized lines see East and Jones (1921), Hayes and Garber (1919), Hayes (1922) and Jones (1920).

about the time the corn was planted, as sporidia develop rapidly in manure and after the corn was up the manure was spread over the field. Beginning when the plants were about three feet high they were sprayed about every ten days in the evening with a water suspension of smut sporidia.

Notes were taken throughout the season for each individual plant on the sort and place of infection, if any. After several years' experience it was concluded that data on infection should be taken at three periods: the first notes at about tasseling time, the next about a month later, and then a note on ear infection at maturity.

The place of plant infection was noted as: S, sucker; E, ear; Eb, ear base; Et, ear tip; L, leaf; Lb, leaf base; M, main stalk; and T, tassel. Multiple infection was noted by Mi and incipient infection by In. The size of the smut boils was estimated by placing them in three groups: S, less than two inches in one or both dimensions; M, of medium size, larger than S and smaller than L; and L, at least 4 inches in diameter in more than one direction. In summing up the notes on total infection the percentage of plants infected, barring incipient infection, was taken as the total percentage infection. Incipient infection was considered to be an indication of resistance because the smut boil starts to develop but does not grow very large and dries up before it ripens. Because of its importance the percentage of ear infection was computed separately.

EXPERIMENTAL RESULTS

Corn is in a highly heterozygous condition under normal conditions. The ease of isolating homozygous strains for resistance or susceptibility to smut depends, in a large measure, on the number of genetic factors involved. If resistance or susceptibility were differentiated by a single factor pair, half of the plants in a field of corn would be homozygous for either resistance or susceptibility and the other half would be in a heterozygous condition. With two factors only one fourth of the plants under normal conditions would be homozygous for various degrees of resistance or susceptibility. The different possible types which could be isolated in a homozygous condition by self-fertilization depends naturally upon the genetic factors involved. The only means of knowing how many factors are involved is to study crosses between types which differ in resistance or susceptibility. The present paper is chiefly a report of the isolation of selfed lines which differ in their heritable mode of reaction to attacks of the smut organism.

Commercial varieties were grown under induced epidemic conditions and individual plants were self-pollinated. Approximately 60 different self-fertilized lines were grown the following year. Some lines were only

slightly infected with smut while others appeared very susceptible. Within several of these self-fertilized lines both infected and non-infected plants were again self-pollinated. The purpose was to determine the comparative susceptibility or resistance of the progeny of infected and non-infected plants of the same selfed line. The results are presented in table 1.

TABLE 1—*A comparison of the progeny of self-fertilized infected and non-infected plants of first generation selfed lines of maize. 1920.*

Variety	1920 selfed strain No.	Infection of parent stalk	Total percentage of infection, 1921	Difference
Minnesota 13	2100	Infected	53.0	— 31.5
		Non-infected	84.5	
Rustler	2400	Infected	26.0	
		Non-infected	20.0	+ 6.0
N. W. Dent	2500	Infected	54.5	
		Non-infected	22.0	+ 32.5
		Non-infected	63.0	— 8.5
N. W. Dent	2700	Infected	47.0	
		Non-infected	36.5	+ 10.5
N. W. Dent	2800	Infected	39.0	
		Non-infected	18.0	+ 21.0
N. W. Dent	3000	Infected	37.5	
		Non-infected	33.0	+ 4.5
Longfellow	3500	Infected	4.5	
		Non-infected	18.0	— 13.5
		Non-infected	29.0	— 24.5
Longfellow	3600	Infected	73.0	
		Non-infected	84.5	— 11.5
King Phillip	4000	Infected	58.5	
		Non-infected	58.0	+ .5
King Phillip	4200	Infected	56.5	
		Non-infected	28.0	+ 28.5
King Phillip	4300	Infected	17.0	
		Non-infected	24.0	— 7.0
Squaw	4400	Infected	29.5	
		Non-infected	38.0	— 8.5
		Non-infected	25.0	+ 4.5
		Non-infected	12.5	+ 17.0

Sixteen comparisons are available of the infection of the progeny of infected and non-infected parent plants of strains of corn which have been selfed for one year. These sixteen comparisons are made within twelve different strains, *i.e.*, in three cases the progeny of an infected parent of a first year self-fertilized strain is compared with two progenies of non-

TABLE 2—*The percentage of plant and ear infection of self-fertilized strains of corn and of the normal varieties from which these strains were isolated.*

Variety	No. years selfed 1923	1923 Cult No.	Total percentage infection					Percentage ear infection				
			1920	1921	1922	1923	Ave.	1920	1921	1922	1923	Ave.
Minnesota No. 13	0	2100	.....	19.0	51.0	12.0	.....	.....	2.1	0.0	0.0	.....
" "	4	700	19.1	58.0	62.8	85.5	56.4	6.4	32.0	26.6	16.8	20.5
" "	4	800	2.3	10.5	65.6	14.0	23.1	0.0	0.0	0.0	2.0	0.5
" "	4	900	9.3	14.0	40.0	21.8	21.3	0.0	0.0	4.0	4.5	2.1
" "	3	2200	.....	23.8	29.3	6.7	.....	.....	.....	0.0	2.3	.....
" "	3	2300	.....	23.8	11.2	6.1	.....	.....	.....	0.0	2.0	.....
" "	3	2400	.....	23.8	31.1	24.1	.....	.....	.....	0.0	4.0	.....
" "	4	2500	2.3	2.0	26.7	22.3	13.3	0.0	0.0	4.2	6.8	2.8
" "	4	2600	2.3	10.5	40.3	27.8	20.2	0.0	0.0	4.3	5.9	2.6
Rustler	0	2700	.....	.....	22.0	23.2	.....	.....	.....	0.0	0.0	.....
"	4	1000	33.3	84.5	100.0	98.0	79.0	5.6	11.2	21.3	28.0	16.5
"	4	1100	3.7	28.5	2.5	12.1	11.7	0.0	14.4	0.0	4.0	4.6
"	4	1200	6.5	2.0	2.2	12.6	5.8	2.2	0.0	0.0	0.0	0.6
"	4	2800	33.3	53.0	88.2	94.7	67.3	5.6	14.1	38.1	20.9	19.7
"	4	2900	3.7	28.5	12.5	35.1	20.0	0.0	14.4	2.1	22.9	9.9
"	4	3000	3.7	16.0	12.5	23.1	13.8	0.0	0.0	0.0	10.5	2.6
"	4	3100	6.5	24.5	29.1	43.1	25.8	2.2	0.0	4.2	0.0	1.6
"	4	3200	11.1	20.0	27.1	12.4	17.7	1.8	0.0	0.0	0.0	0.5
"	4	3300	11.1	20.0	49.6	18.4	24.8	1.8	0.0	4.2	4.0	2.5
N. W. Dent	0	3400	.....	.....	61.4	53.0	.....	.....	.....	0.0	4.1	.....
"	4	3500	22.6	63.0	95.7	75.2	64.1	5.7	6.2	2.2	15.3	7.4
"	4	3600	22.4	36.5	33.4	32.2	31.1	10.3	14.2	10.5	2.3	9.3
"	4	3700	22.4	47.0	89.9	92.6	63.0	10.3	4.1	12.4	24.0	12.7
"	4	3800	8.5	18.0	38.0	15.8	20.1	2.1	4.0	0.0	0.0	1.5
"	4	3900	8.5	18.0	59.4	49.3	33.8	2.1	4.0	0.0	26.2	8.1
"	4	4000	5.0	42.0	42.0	16.6	26.4	1.7	2.0	0.0	0.0	0.9
"	4	4100	5.0	24.0	32.4	22.4	21.0	1.7	6.0	2.0	13.9	5.9
"	4	4200	20.0	33.0	75.0	94.8	55.7	6.7	4.0	7.5	36.1	13.6
Minnesota No. 23	4	4300	20.0	56.5	81.7	77.1	58.8	4.0	36.8	46.7	59.7	36.8
" "	4	4400	50.0	80.5	63.4	77.6	67.9	17.9	18.4	4.0	8.2	12.1
" "	4	4500	65.6	88.0	93.8	56.2	75.9	27.9	12.5	2.1	4.7	11.8
" "	4	4600	38.7	30.0	59.3	29.8	39.5	9.7	2.1	10.3	2.7	6.2
Longfellow	0	4700	.....	.....	38.9	32.4	.....	.....	.....	6.1	20.6	.....
"	1	4800	.....	.....	.....	74.4	.....	.....	.....	.....	48.9	.....
"	4	4900	15.5	18.0	44.9	6.0	21.1	11.1	0.0	0.0	0.0	2.8
"	4	5000	32.6	73.0	83.7	65.9	63.8	16.3	35.4	20.9	26.5	24.8
"	4	5100	14.7	14.0	2.3	12.4	10.9	5.9	14.2	0.0	6.2	6.6
King Phillip	0	5200	.....	.....	43.2	41.7	.....	.....	.....	4.5	15.0	.....
"	4	5300	31.0	58.0	66.8	97.5	63.3	22.4	41.7	51.3	95.0	52.6
"	4	5400	31.0	58.0	34.7	80.0	50.9	22.4	41.7	25.6	70.0	39.9
"	4	5500	31.0	58.5	70.1	57.1	54.2	22.4	39.6	37.1	48.0	36.8
"	4	5600	37.5	74.5	61.3	41.8	53.8	12.5	12.9	4.6	15.7	11.4
"	4	5700	24.1	24.0	36.5	64.7	37.3	0.0	14.0	12.2	46.9	18.3
"	4	5800	24.1	17.1	38.8	27.0	26.8	0.0	4.2	2.1	8.0	3.6
Squaw	0	5900	.....	.....	54.5	39.4	.....	.....	.....	15.2	14.6	.....
"	4	6000	21.5	12.5	80.0	80.3	48.6	7.7	8.2	8.9	12.8	9.4
"	4	6100	21.5	25.0	34.3	42.6	30.9	7.7	4.3	8.5	5.0	6.4
"	4	6200	21.5	29.5	38.5	69.7	39.8	7.7	0.0	8.7	28.1	11.1
1–9–1, etc.	14	1700	12.9	51.5	60.9	67.3	48.2	1.6	0.0	0.0	0.0	0.4
1–6–1, etc.	14	1800	18.4	.....	48.0	71.7	.....	8.6	.....	5.0	37.0	.....
69–6, etc.	14	1600	29.9	21.0	34.5	24.2	27.4	9.5	21.0	15.6	0.0	10.4

infected plants of the same strain. If the progeny of the infected parent plant is more severely infected than the progeny of the non-infected parent plant the difference is presented in the table as a plus difference. When the progeny of the non-infected plant is the more severely infected, the difference is presented as a minus difference. There were 7 minus differences and 9 plus differences. The sum of the plus differences was 125 and of the minus differences 105. These results prove that under the conditions of the experiment it makes little difference whether a selfed plant is infected or not. The important fact is the hereditary characters of the strain. In table 2 data are presented on the total percentage of smut infection of the self-fertilized strains. The two strains obtained from Jones, of Connecticut, were rather susceptible under the conditions of the experiment, although not as susceptible as some selfed strains produced under induced epidemic conditions. The long time self-fertilized flint strain, 69–6, etc., apparently bred true for an intermediate degree of infection, which indicates that moderately susceptible strains can be secured.

Probable errors for total percentage of smut infection were computed in 1920 and 1921 by the pairing method. In brief, this method consists of obtaining the deviation in percentage of smut infection of each two pairs of plots of the various selfed lines. By dividing the average deviation in per cent for all pairs by the $\sqrt{n}$ where n represents the number of systematically distributed plots, a probable error for the method of test has been computed. This method was suggested by Wood and Stratton (1910) and it gives on the average slightly lower probable errors than the use of other methods.

For 1922 and 1923 the probable errors were calculated by the following formula:

$$\text{P.E.} = \pm .6745 \frac{\sqrt{\frac{(\text{dev. in } \%)^2}{n}}}{\sqrt{N}}$$

In this formula Σ = summation, dev. in % = deviation in percentage infection of each plot of a selfed strain from the mean of all the plots of that strain, N = number of plots of each strain and n = number of deviations in percentage. The use of this formula gives probable errors of nearly the same magnitude as those obtained from check plots (Hayes, 1923).

Probable errors computed by these methods furnish an average probable error in per cent for the particular experiment. They do not take into account the possible condition that the reaction of some strains is more variable to a particular environmental condition than that of others. Probable errors are presented in table 3.

TABLE 3—*Probable errors in percentage for smut infection.*

Year	Probable error in per cent for percentage smit infection of each selfed line
1920	6.1
1921	7.4
1922	13.3
1923	15.2

The average probable error for the 4 years was computed by the formula $\frac{1}{N}\sqrt{a^2 + b^2 + c^2 + n^2}$ where N represents the number of things averaged and a, b, $\cdots$ n represent the separate probable errors.

By the use of this formula the average probable error in percentage was found to be 5.6 per cent. This probable error was used to obtain average probable errors of smut infection percentages in selfed lines. Certain of the selfed lines have appeared to be homozygous for their smut reaction and have given consistently high or low percentage infection for each of the four years while other lines show evidence of being heterozygous. The average ear infection and the average total infection of lines which appear to be homozygous is given in table 4.

TABLE 4—*Summary of selfed strains which appear to be homozygous for smut reaction for the period 1920–1923 inclusive.*

Variety	1923 Strain No.	Total percentage infection	Percentage of ear infection
Minnesota 13	700	56.4 ± 3.1	20.5
"	2500	13.3 ± 0.7	2.8
Rustler	1000	79.0 ± 4.4	16.5
"	2800	67.3 ± 3.8	19.7
"	1200	5.8 ± 0.3	0.6
"	3000	13.8 ± 0.8	2.6
"	3200	17.7 ± 1.0	0.5
N. W. Dent	3500	64.1 ± 3.6	7.4
"	3700	63.0 ± 3.5	12.7
"	4200	55.7 ± 3.1	13.6
"	4000	26.4 ± 1.5	0.9
Minnesota 23	4400	67.9 ± 3.8	12.1
"	4500	75.9 ± 4.3	11.1
Longfellow	5000	63.8 ± 3.6	24.8
"	5100	10.9 ± 0.6	6.6
King Phillip	5300	63.3 ± 3.5	52.6
"	5500	54.2 ± 3.0	36.8

Comparing the two strains 700 and 2500 of Minnesota 13, a difference for total infection of 43.1 ± 3.2 is obtained. The chances are very great that this difference is significant. Certain strains appear to be homozygous for an intermediate type of susceptibility. Thus Northwestern Dent strain

4000 had an average infection of 26.4 ± 1.5 while Rustler Dent strain 1200 had an average infection of 5.8 ± 0.3. Strains 4000 and 1200 differ in smut reaction by the percentage infection of 20.6 ± 1.5. The chances are also very great that this is a significant difference.

The place of infection on the plant is a strain characteristic. Thus strain 1–9–1, etc., which was resistant under Connecticut conditions, produced an average total infection of 48.2 per cent and an average ear infection of only 1.5 per cent, while strain 69–6, etc., had only an average total infection of 24.2 per cent, but an average ear infection of 10.4 per cent. One strain which was under observation in the Plant Breeding Nursery for several years produced large smut boils near the base of the plant on a large percentage of the plants. This tendency was noted from year to year. Some strains are severely infected in the tassel and scarcely ever infected in other parts of the plant, while still other strains, such as King Phillip, Strain 5300, produced a very high percentage of ear infection. The extent to which total infection is inherited from year to year is demonstrated by the calculation of correlation coefficients for smut infection in different seasons. The correlation coefficients would presumably be larger in homozygous than heterozygous material. Coefficients are presented in table 5 and table 6 for the extent to which total percentage infection one year is correlated with total percentage infection in other years and likewise the extent to which ear infection one year is correlated with ear infection in other years.

TABLE 5—*Correlation in selfed strains of maize between total percentage infection of the strains in one year with the percentage infection in other selfed generations.*

Generations correlated	No. of selfed lines	Correlation coefficient
1st and 2nd	58	.696 ± .046
2nd and 3rd	40	.642 ± .063
3rd and 4th	38	.712 ± .054
2nd and 4th	38	.695 ± .057

These coefficients are uniformly large and prove that the degree of smut infection in selfed lines of maize is dependent upon strain characters.

As has been noted, smut infection on the ear is probably more detrimental to the yielding ability of the corn plant than infection of other parts of the plant. Accordingly correlation coefficients were computed for the purpose of learning to what extent the percentage of ear infection was a strain characteristic. The results of this study are presented in table 6.

These correlations coefficients are somewhat larger than those obtained when total percentage infection was studied. They prove that the selfed lines which differ in reaction to smut can be isolated in a very short time.

TABLE 6—*Correlation in selfed strains of maize between percentage of ear infection in one season with the percentage infection in other selfed generations.*

Generations correlated	No. of selfed lines	Correlation coefficient
2nd and 3rd	40	.859 ± .028
3rd and 4th	38	.761 ± .046
2nd and 4th	38	.765 ± .045

RELATION BETWEEN YIELD AND PERCENTAGE OF SMUT INFECTION

The ears of twenty stalks from each selfed line were harvested for the purpose of learning the comparative vigor of strains. It is realized that a yield test made on this basis is not particularly accurate; but it was hoped that such a test would give some indication of comparative yielding ability. The yields of each group of selfed strains of a variety were averaged separately and a yield index was computed by dividing the average yield of a strain by the average yield of other strains of the same variety which had been self-fertilized for the same length of time. The extent to which yield for one year is correlated with that for another year as computed by this method of obtaining yields may be determined by the use of the correlation coefficient (see table 7).

TABLE 7—*Correlation between yields of third and fourth year selfed lines in 1922 and 1923.*

Yield index, 1922, 3rd year selfed.	Yield index—1923. Fourth year selfed													
	18	37	56	75	94	113	132	151	170	189	208	227	246	Total
56	1		1	1										3
75			1	1	1	1								4
94	1		3	1	1	2	2		1					11
113	1		2	3		4		1					1	12
132	1			1		2				1				5
151											1			1
	4	—	7	7	2	9	2	1	1	1	1	—	1	36

$r = +.351 \pm .099$.

Since the method of obtaining yield data is necessarily inaccurate, a high degree of correlation between smut and yield cannot be expected. However, some idea as to whether smut infection influences yielding ability may perhaps be obtained by correlating yield with the percentage of ear infection.

Correlation coefficients are presented in tables 8 to 10 inclusive, which represent the correlation between yield in the 2nd, 3rd, and 4th selfed generation and reaction to smut in these same generations. Each of the three

correlation coefficients is negative, although one is lower than its probable error. The two other coefficients are respectively 2.7 and 2.4 times their probable errors and the chances that they are significant are 13.6:1 and 8.5:1 respectively.

TABLE 8—*Correlation between the percentage of smut infection of the ear and yields of lines selfed for two years. (1921).*

Per cent smut ear infection. 1921.	Yield index—1921.										Total
	68	75	82	89	103	110	117	131	138	159	
2	1	2	3			4		2	2	1	15
7			1	1							2
12	1	1	2		1	1	1			1	8
17									1		1
32							1				1
37			1					1			2
42				1		1					2
	2	3	7	2	1	6	2	3	3	2	31

$r = -.0.003 \pm .0.116.$

TABLE 9—*Correlation between the percentage of smut infection of the ear and yields of lines selfed for three years. (1922).*

Per cent smut ear infection. 1922.	Yield index—1922.														Total
	54	61	68	75	82	89	96	103	110	117	124	131	138	145	
2				3	1	4	2	3	3	3		2		1	22
7		1							1			1			3
12					2			1	1	1					5
22		1											1		2
27		1	1												2
37								1			1				2
47	1														1
52						1									1
	1	3	1	3	3	5	2	5	5	4	1	3	1	1	38

$r = -0.274 \pm 0.101.$

REACTION OF F_1 CROSSES BETWEEN SELFED LINES

In 1923 certain F_1 crosses and their parents were compared for smut reaction. The results are presented in table 11.

TABLE 10—*Correlation between the percentage of smut infection of the ear and yields of lines selfed for four years. (1923).*

Per cent smut ear infection, 1923.	Yield index—1923.														
	18	37	56	75	94	113	132	151	170	189	208	227	246	265	Total
5	1		3	2	2	3	2	1		1	1		1	1	18
14	1		2	2		1									6
23	1			1		4									6
32			1	2											3
50						1			1						2
59	1														1
68				1											1
95			1												1
	4	—	7	8	2	9	2	1	1	1	1	—	1	1	38

$r = -0.248 \pm 0.103$

TABLE 11—*Reaction of F_1 crosses and their parents to attacks of Ustilago zeae.*

Variety	Parent line or F_1 cross	Generation selfed or F_1	Total per cent smut	Per cent ear smut
Minnesota 13	800	4	14.0	2.0
	900	4	21.8	4.5
	800 × 900	F_1	8.6	0.0
Minnesota 13	700	4	85.5	16.8
	800 × 700	F_1	45.1	12.2
Rustler	1000	4	98.0	28.0
	1200	4	12.6	0.0
	1000 × 1200	F_1	25.0	4.4
Rustler	1100	4	12.1	4.0
	1100 × 1200	F_1	3.0	0.0
Longfellow	1300	4	7.4	0.0
	1400	4	67.1	30.6
	1300 × 1400	F_1	60.7	17.8
Longfellow	1500	4	69.1	13.0
	1300 × 1500	F_1	24.7	10.9

In the study made by Jones the F_1 hybrid approached the condition of the immune parent but dominance of immunity was not perfect. In the F_1 crosses reported here two opportunities are given to compare F_1 crosses between resistant selfed lines with their parents. In both cases the cross is slightly more resistant than either parent. These crosses are (800 × 900) and (1100 × 1200) which are crosses between selfed strains of Minnesota No. 13 and Rustler respectively. Four F_1 crosses between resistant and susceptible strains have been compared with their parents. One cross,

800 × 700, is almost exactly intermediate in percentage of infection. In this case the total percentage of infection of the cross is 45.1 and of the average of the parents 49.8. Two crosses (1000 × 1200) and (1300 × 1500) show a partial dominance of resistance while the cross of 1300 × 1400 is nearly as susceptible as the susceptible parent. In general, then, F_1 crosses between susceptible and resistant selfed strains of corn are intermediate in type of reaction to smut.

SUMMARY

1. The most promising means of controlling corn smut, caused by *Ustilago zeae*, appears to be the development of resistant varieties.

2. Ear infection is more serious than infection of other parts of the plant. Flint varieties appear to be somewhat more susceptible, particularly to ear infection, than dent varieties.

3. A normal variety of corn is in a highly heterozygous condition. Selection in self-fertilized lines appears to be the most promising means of isolating smut-resistant strains of corn.

4. Apparently it is easy to isolate self-fertilized lines which differ in their inherited reaction to smut, which indicates that only a few genetic factors are involved in resistance or susceptibility.

5. The infection in one generation was correlated with the infection in succeeding generations. The correlation coefficients were uniformly large which leads to the conclusion that resistance or susceptibility is a strain characteristic.

6. The localization of the smut on the plant appears also to be a strain characteristic. Some selfed strains were uniformly infected on one of the lower nodes of the stalk, other strains produced a high percentage of ear infection and still others were infected chiefly in the tassel. Some of the very susceptible strains were infected rather generally in all parts of the plant.

7. F_1 crosses between resistant self-fertilized strains were more resistant than either parent while F_1 crosses between resistant and susceptible strains produced an intermediate type of infection.

8. Resistance and susceptibility appear to be conditioned by genetic factors. This leads to the conclusion that the susceptible strains of a variety may be isolated and discarded by means of selection in self-fertilized lines and that resistant strains can be isolated and then used to build resistant varieties.

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NOTES ON THE LIFE HISTORY OF THE SNAPDRAGON RUST, PUCCINIA ANTIRRHINI DIET. & HOLW.

E. B. MAINS[1]

The complete life history of the snapdragon rust has never been reproduced. Under greenhouse conditions, at least, the uredinial stage alone is sufficient to maintain the rust, if snapdragons are being propagated by cuttings. If snapdragons are grown from seed, the propagation of the rust by urediniospores can be prevented, care being taken to destroy all rusted snapdragons some time previous to the sowing of the seed. That the urediniospores are not capable of carrying the rust over long periods has been shown by Doran,[2] who found very little germination after four weeks and none at the end of eight weeks. Out of doors the rust will survive the winter in the uredinial stage whenever rusted plants live over. However, during severe winters, unprotected plants are usually killed in this region.

Another spore stage of the snapdragon rust is known, the teliospore. This is not produced so abundantly as is the urediniospore. Doran states that occasionally the teliospores may be found outdoors in November, while in the greenhouse they are formed only when the plants are gradually deprived of water. Both Doran (*l. c.*, 2) and Peltier[3] were unsuccessful in their attempts to germinate the teliospores of this rust. Peltier states that all efforts to germinate the teliospores failed, alhough they were subjected to alternate wetting and drying, to high and low temperatures, to various outdoor conditions through the winter and summer, and were tested by the gelatine plate method under different conditions. Doran used fresh material, dried material, teliospores produced under glass and produced outside and teliospores overwintered outside. They were tested to 7°, 10°, 12° and 20° C., but in no case was germination obtained. Doran suggests that these spores may be able to germinate only within a very narrow range of temperatures. Hockey[4] has recently reported the successful germination of the teliospores of *Puccinia antirrhini*. The teliospores used were obtained from greenhouse plants. Hanging drops of teliospores gave 14 per cent germination from material which had been frozen for 1 day

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station.

[2] Doran, W. L. Rust of Antirrhinum. Massachusetts Agric. Exp. Sta. Bull. 202. *p. 39–66. 3 pl.* 1921. Literature cited, p. 65–66.

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[4] Hockey, J. F. Germination of Teliospores of *Puccinia Antirrhini*. 13th Ann. Rept. Quebec Soc. Protect. Plants, Ann. Rept. 1920–1921: 54–57. Bibliography, p. 57.

outside on a compost heap in January, 1921. As high as 22 per cent germination was obtained after the teliospores had been exposed outside for 14 days. Spores from a plant frozen seven days and kept at room temperature four days and then frozen again for four weeks gave only 2 per cent germination. Seedling plants of snapdragon inoculated with germinating teliospores bearing basidiospores gave no infection, while similar plants inoculated with urediniospores gave characteristic uredinia.

In light of the above, it seems desirable to record the results obtained in this laboratory during the past five years. As part of the investigations being carried on here with the rust of snapdragons, the writer has been considerably interested in obtaining germination of the teliospores in order to determine their function in the propagation and biology of the rust. Several important questions depended for their answer on cultures made with teliospores. Is the rust autoecious or heteroecious? Whether the teliospores may directly infect snapdragon, serving as resting spores to carry the rust over adverse conditions such as winter out of doors, summer in the greenhouse, or whenever the continuous propagation of the rust by urediniospores is interrupted, or whether an alternate host is necessary before the teliospores are of importance, are some of the points to be settled. If the rust is autoecious, then the teliospores are a constant source of danger and the effect of conditions during and following formations on the viability of the teliospores, the length of the dormancy period, conditions determining and favoring germination, are all questions of considerable importance. If, on the other hand, the rust is heteroecious, these questions are of importance only when the alternate host is present and consequently the identity and occurrence of such a host would become the most important question. If the rust is autoecious, it may either be a brachy-Puccinia or an eu-Puccinia. If a brachy-Puccinia, infection from teliospores would give pycnia accompanied by uredinia; if an eu-Puccinia, pycnia accompanied by aecia. If heteroecious, no infection of course would be obtained on snapdragon and the rust must be an eu-Puccinia producing pycnia and aecia on the alternate host. Also from a taxonomic point of view these questions are important since in the classifications of the rusts proposed by Arthur[5] and by Sydow[6] the number of spore forms and the heteroecious or autoecious character are used to determine the generic position of the species of rusts. Without germination of the teliospores these questions could not be answered. It might

[5] Arthur, J. C. Eine auf die Struktur und Entwicklungs geschichte begründete Klassifikation der Uredineen. Résult. Sci. Congr. Bot. Vienne **1905**: 331–348. 1906.

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be deduced since *Antirrhinum majus* belongs to the Scrophulariaceae, that the rust would likely be autoecious, judging by other species of Puccinia on this and related families. Granting that the germination of teliospores occurs under the cultural conditions used with snapdragon, the absence of aecia, which would not likely be overlooked, points to a brachy-Puccinia, the primary uredinia of such a rust being easily mistaken for the uredinia ordinarily found. However, such deductions are erroneous, as is shown by the results obtained. These, taken with those obtained by Hockey, while not actually settling all the questions involved, throw some light on the function of the teliospore in the propagation and life history of the rust.

RESULTS OF STUDY

1918. The first collection of telia used in this study was made on August 10, 1918, from heavily rusted plants near a local greenhouse. Only a small amount of telia was found and these were tied in a coarse cheese cloth bag and hung outside to winter. No test was made for germination until April 24, 1919, when hanging drops of the overwintered spores were made. No germination was noted at this time and on account of the pressure of other culture work no more tests were made that year.

1919. On December 12, 1919, teliospores were found upon old plants of snapdragon in the greenhouse, both dead and green stems. A small amount of teliospores from each was collected and tested for germination in hanging drops on that date. No germination was obtained. Each collection was then overwintered out of doors as before. Hanging drops of the teliospores of each collection were made March 24, April 2, April 15, April 28 and May 11, 1920. On April 15, a very few spores showed germination from the material from green stems. No typical basidia with basidiospores were formed. Some of the teliospores including those in the hanging drop were sown on young rust-free snapdragons, but without obtaining infection. Germination was not obtained in any other tests. It was felt that no conclusions could be drawn from this on account of the apparent lack of basidiospore formation.

1920. In the fall of 1920, some very fine material was received from Mr. E. Bethel from Monrovia, California. This consisted of a large number of snapdragon plants bearing abundant teliospores. In addition Mr. Bethel sent a quantity of *Adenostegia filifolia* also bearing plenty of teliospores. The rust on the latter host has been described as a distinct species, but in the North American Flora, Arthur[7] considers it as belonging to *Puccinia antirrhini*. The material was placed out to winter as before and on December 14, 1920, hanging drops of each showed a very heavy germination, the

[7] Arthur, J. C. Uredinales. North American Flora 7: 594–5. 1922.

teliospore masses being white with the basidia, and a great abundance of basidiospores was formed. Teliospores from both of these collections, including those from the hanging drops were sown on four young rust-free snapdragons without obtaining infection. On April 4, 1921, the spores were again tested, after having been outdoors all winter, those from snapdragon showed a slight germination, while those from Adenostegia showed no germination. Another test April 19, gave no germination in either case.

1921. An attempt to repeat this work was made in 1921. Snapdragons bearing teliospores were obtained in the fall from Professor Blasdale from Berkeley, California. This material was placed out doors to winter as before. It was tested for germination October 18, November 3, November 17, December 3 and December 20, 1921, and January 24, 1922, without showing germination and was in consequence not sown. In addition, in the fall of 1921, a small amount of teliospores was obtained from a local planting. These were brought into the greenhouse and tested for germination November 1, no germination resulting. They were then left on a greenhouse bench until December 13, when a slight amount of germination was noted. However, when sown on young snapdragons they gave no infection.

1922. In the fall of 1922, an abundance of material for culture was obtained from a heavily infected planting at the Experiment Station. A quantity of this was brought into the greenhouse on December 21 and used to mulch 8 pots of young, rust-free snapdragons. The teliospores at that time showed no germination. When tested again on January 24, 1923, they gave a very vigorous germination with abundant basidiospore production. The mulched plants were then placed in an incubation chamber and the humidity brought up to saturation. The germinating spores from the hanging drops as well as some from the mulch were placed on the leaves and stems of the plants. However, no infection was obtained. Volunteer plants which appeared in the field in the spring of 1923 where the rusted snapdragons bearing teliospores had been piled up showed no infection until rusted plants from the greenhouse were set out.

In all the above work the young plants upon which the teliospores were sown were later inoculated with urediniospores of the rust and heavy infection was obtained showing that the plants used were susceptible to the rust. The tests for germination were made in hanging drops left over night at the temperature prevailing in the greenhouse which varied from 10–20° C. The plants were inoculated under these same conditions.

DISCUSSION

The results obtained by Hockey (*l. c.*, 4) and the writer show that the teliospores of *Puccinia antirrhini*, under some conditions at least, are capable of germinating. The results obtained by Peltier and Doran show

that this is not to be expected under all conditions. Doran has suggested the possibility that the teliospores are able to germinate only through a narrow range of temperatures. Since the teliospores tested by the writer were subjected to a variation of temperature, it can not be said that such a possibility is eliminated. However, it seems more likely that conditions to which the teliospores are subjected during and following formation are of more importance. Doran has noted that the amount of teliospore formation in the greenhouse is considerably influenced by the treatment given to the snapdragon plants. It would seem likely that this would also have an influence on their viability. It is interesting to note in this connection that the most vigorous germination of teliospores obtained by the writer was from the materal having the greatest teliospore formation. Erikson[8] has noted a somewhat similar situation for teliospores of *Puccinia coronata* f. sp. *Agrostis*. That environmental conditions may have a decided effect on the teliospores themselves is indicated by the observations of Stakman, Kirby and Thiel[9] who report that in the southern United States teliospores of *Puccinia graminis* are not able to infect barberry. In the case of *Puccinia antirrhini* it is evident that some seasons and in some localities teliospores may be produced in great abundance. Since out of the seven collections made of teliospores of *Puccinia antirrhini* studied at Lafayette, Ind., five gave germination, the teliospores are a factor which must be taken into consideration in connection with the rust of snapdragon.

Although the results obtained by sowing teliospores of the rust are not all that could be desired, negative results never being as satisfactory as positive, nevertheless they furnish some interesting information concerning a number of the questions involved. The lack of infection when germinating teliospores were sown on snapdragon plants indicates that the rust is heteroecious and eliminates them as a source of danger in the spread of the rust unless the alternate host is present. Since all heteroecious rusts produce pycnia and aecia on the alternate host, it must follow that *Puccinia antirrhini* is a long cycled rust, *i.e.*, having all spore forms.

What the aecial host of *Puccinia antirrhini* may be, is an interesting question. The rust does not possess any marked peculiarity of structure or marking which greatly distinguish it from many other species of the genus Puccinia. Consequently it is almost a hopeless task to attempt to find the correlated short-cycled Puccinia and thus determine the genus of the alternate host.[10] It is very doubtful if the aecial host is to be found throughout

[8] Eriksson, Jakob. Ueber die Dauer der Keimkraft in den Wintersporen gewisser Rostpilze. Centralblatt. Bakt. Parasitenk. u. Infektionskrankh. **4**: 376–388. 1898.

[9] Stakman, E. C., Kirby, R. S., and Thiel, A. F. The regional occurrence of *Puccinia graminis* on barberry. Phytopathology **11**: 39–40. 1921 (Abstract).

[10] For discussion and examples of the use of this method of correlation see Proc. Ind. Acad. Sci. **1921**: 133–135. *1 pl.* 1922.

the present range of the rust and in consequence attempts to find an association of the stages is likely to be largely wasted effort. All evidence indicates that this rust did not originally occur on snapdragon. *Antirrhinum majus* is a native of the Mediterranean region, but the rust is unknown there. *Puccinia antirrhini* was first discovered by Blasdale[11] on cultivated snapdragon at San Leandro, California, in 1896 and was described by Dietel and Holway[12] in 1897 from a collection made by Blasdale at Berkeley, California. Outside of California and Oregon, the rust was not noted until about 1913 when it appeared in a serious way in the vicinity of Chicago, Ill. Since then it has been spread, until it is now found in twenty of the states of the United States, three provinces of Canada and in Bermuda. Apparently, therefore, the rust originated in Califorina, probably from some native species of Antirrhinum. Blasdale reports being able to infect *Antirrhinum vagans* Gray with the rust from snapdragon and considers collections of the rust made upon *A. nuttalianum* Benth. and *A. virga* Gray as being *Puccinia antirrhini*. Two other rusts very close to *P. antirrhini* have been described from California, *P. adenostegiae* Arth. on *Adenostegia pilosa* (A. Gray) Green, and *A. rigida* Jepson and *Puccinia cordylanthi* Blasdale on *Adenostegia filifolia* (Nutt.) Abrams (*Cordylanthus filifolius* Nutt.). Blasdale considers each a distinct species, while Arthur has brought them together under the name of *Puccinia antirrhini*. These conclusions were reached without cross inoculations so that it is not certain that Adenostegia species are hosts for the snapdragon rust, but they should be kept in mind.

It would seem as if location and observation of the rust on these native species would be the most likely method of obtaining clues as to the aecial host. Apparently the California species of Antirrhinum are not abundant and easily accessible. Both the Antirrhinums and Adenostegia species are found on dry hills. This, taken with the fact that the teliospores apparently germinate most abundantly soon after maturity, may possibly indicate germination taking place and the infection of the alternate host occurring with the beginning of the fall rains. This also suggests a possibility that the aecial stage is systemic in some winter annual or a perennial in a manner similar to *Puccinia eatoniae* which produces its telia on species of Eatonia, the teliospores germinating in the fall, infecting *Ranunculus abortivus*, producing a systemic mycelium from which aecia are formed the next spring.

SUMMARY

1. Germination of the teliospores of *Puccinia antirrhini* has been ob-

[11] Blasdale, W. C. A Preliminary List of the Uredinales of California. Univ. Calif. Publ. Bot. 7: 101–157. 1919.

[12] Dietel, P. Einige neue Uredineen. Hedwigia 36: 297–299. 1897.

tained from five out of seven collections studied and during four of the five years tests have been made.

2. Sowings of the germinating teliospores on young, rust-free snapdragons did not give infection in any case.

3. This would indicate that the rust is heteroecious and has pycnia and aecia on an alternate host.

4. It is considered that the most likely method of discovering this host is by observations in California on native species of Antirrhinum in localities where they are infected with the rust.

PHYTOPATHOLOGICAL NOTES

Resistance of sorghum to loose and covered smuts.—The writer has carried out an experiment to determine the reaction of certain varieties of sorghum to *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter. The seed of ten varieties of sorghums were received from Dr. George M. Reed, Brooklyn Botanic Garden, Brooklyn, N. Y., and separate lots inoculated with the spores of the two smuts and sown at Modi Bag Agricultural College, Poona, India.

Five of the varieties used were reported by Reed (Mycologia, **15:** 132–143, May, 1923) as resistant and the remaining five as susceptible to both *Sphacelotheca sorghi* and *S. cruenta* in his experiments. My results are given in the following table:

	Sphacelotheca Sorghi		*Sphacelotheca Cruenta*	
	No. of heads	Per cent Infected	No. of heads	Per cent Infected
Milo, Dwarf	200*	0	200	0
Milo, Standard	200*	0	200	0
Feterita	370	.2	325	.3
Feterita, Spur	200*	0	200	0
Milo, White	200*	0	200	0
Kafir, Sunrise	205	9.2	240	2.05
Kafir, Blackhull	180	18.1	180	4.7
Kafir, Red	165	50.1	307	13.5
Kafir, Dawn	216	40.7	225	23.5
Shallu	140	42.1	178	36.6

* Number of heads estimated.

These results are quite similar to those reported by Reed. The resistant varieties have shown their resistance in my experiments and the susceptible varieties have been more or less severely infected. My earlier reports on the susceptibility of Dwarf Milo (Phytopathology, **11:** 252, 1921) was a mistake due to the fact that the variety used turned out not to be Dwarf Milo. —G. S. KULKARNI.

British Association for the Advancement of Science.—The annual meeting of the British Association for the Advancement of Science in 1924 will take place in Toronto, Canada, from Wednesday, August 6, to Wednesday, August 13. The section in which plant pathologists will be most interested is Section K (Botany), of which Professor V. H. Blackman is the President and F. T. Brooks, 31 Tension Avenue, Cambridge, England, is the Recorder. Professor J. H. Faull and Professor R. B. Thompson, both of the University of Toronto, are chairman and secretary respectively of the local committee for this section. The botanical program is not available at this time but notice has been given of a joint session of the Botany and Zoology Sections on *The Species Concept*. It is hoped that many members of the American Phytopathological Society will plan to attend these meetings.—R. J. HASKELL.

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